TIANGEN®

EndoFree Mini Plasmid Kit II

For purification of ultrapure plasmid DNA with high yield

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EndoFree Mini Plasmid Kit II

(Spin Column)

Cat. no. 4992422

Kit Contents

Contents	4992422 50 preps
Buffer BL	30 ml
Buffer P1	30 ml
Buffer P2	30 ml
Buffer P4	30 ml
Buffer PD	30 ml
Buffer PW	15 ml
Buffer TB	15 ml
RNase A (10 mg/ml)	300 μl
Filtration Columns CS	50
Spin Columns CP4	50
Collection Tubes 2 ml	100
Handbook	1

Storage

EndoFree Mini Plasmid Kit II can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers to 37°C before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After adding RNase A, Buffer P1 is stable for 6 months at 2-8°C.

Introduction

EndoFree Mini Plasmid Kit II uses unique silica membrane technology which can specifically adsorb plasmid DNA efficiently. Meanwhile, this kit also uses Buffer P4 and Filtration Column CS to get rid of contaminants like endotoxin and protein compounds effectively. The whole experimental procedure of plasmid DNA extraction could be finished within 1 h. The following protocol is for the isolation of plasmid DNA from overnight culture of *E. coli* in LB (Luria-Bertani) medium. The yield and quality of isolated plasmid DNA depend on the cell strain, cell culture condition, lysis of cells, copy number of plasmid, the stability of plasmid and the type of antibiotics.

Plasmid DNA prepared by EndoFree Mini Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, PCR, sequencing, ligation, and transfection to cells.

Yield

Plasmid type	Volume of cell culture	Yield	Plasmid
Low copy	5-15 ml	5-25 μg	pBR322, pACYC, pSC101 SuperCos, pWE15
High copy	5-15 ml	15-70 μg	pTZ, pUC, pBS, pGM-T

Important Notes **Please read before use.**

1. Add the provided RNase A solution to Buffer P1 (use 1 vial RNase A per bottle Buffer P1), and store at 2-8°C.
2. Add ethanol (96-100%) to Buffer PW before use (check bottle label for volume).
3. Check Buffer BL, P2 and P4 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes.
4. Avoid direct contact of Buffer P2 and P4, immediately close the lid after use.
5. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-30°C), the speed should be 12,000 rpm (~13,400 × g).

6. The amount of extracted plasmid is related to cells concentration and plasmid type. If working with low copy or large plasmid (>10 kb), it may be beneficial to increase culture volume and to increase Buffer P1, P2, and P4 in proportion. Warm the Buffer TB at 65-70°C before use. Prolong adsorption and elution time properly to increase extraction efficiency.
7. Use Buffer BL to treat spin columns could activate silica membrane at maximum degree and higher yield.
8. After treated with Buffer BL, use the Spin Column soon, since long-term placement may affect the purifying effect.

Protocol:

1. Column equilibration: place a Spin Column CP4 into 2 ml collection tube and add 500 μl Buffer BL to Spin Column CP4. Centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through, and set Spin Column CP4 back into the same collection tube.
2. Transfer 5-15 ml overnight bacterial culture into a centrifuge tube and harvest cells by centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and then remove all the supernatant by pipetting.

   Note: For large volume of bacterial cells, please harvest bacterial cells to one tube by several centrifugation steps. Too much bacterial cells will lead to incomplete lysis and further reduce plasmid yield.
3. Resuspend pelleted bacterial cells in 500 μl Buffer P1 **(Ensure that RNase A has been added)**, mix by vortex or pipetting.

   Note: No cell clumps should be visible after resuspension of the pellet, since incomplete resuspension will reduce the yield and purity of isolated plasmid DNA.
4. Add 500 μl Buffer P2 and mix thoroughly by gently inverting the tube 6-8 times.

   Note: Mix by gently inverting the tube. Do not vortex, as this will

result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and clear. Do not allow the lysis reaction to proceed for more than 5 min. If the solution won't turn clear, please reduce the amount of cells.

5. Add 500 μl Buffer P4, and mix immediately and thoroughly by gently inverting 6-8 times, white precipitate should be formed in solution at this point. Incubate the solution at room temperature (15-30°C) for 10 min, centrifuge at 12,000 (~13,400 × g) for 10 min, precipitate should be formed at the tube bottom.

   Note: To avoid localized precipitation, mix the lysate thoroughly and immediately after addition of Buffer P4. If there is still small white precipitate in the supernatant, please centrifuge again.

6. Transfer the supernatant from step 5 to Filtration Column CS **(place the Column in a 2 ml collection tube)** separately, centrifuge for 2 min at 12,000 rpm (~13,400 × g). Keep the flow-through in 2 ml microcentrifuge tubes (not supplied in kit).

7. Add 0.3 volume isopropanol **(excess amount of isopropanol may lead to RNA contamination)** to the cleared filtrate, mix by inverting and then transfer all solution to the Spin Column CP4 **(place the Spin Column CP4 in a 2 ml collection tube)**.

   Note: There will be a loss on solution during the filtration process, please add appropriate amount of isopropanol to the solution according to the amount of loss. The capacity of the Spin Column CP4 is 700 μl, please load solution to the column separately.

8. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min under room temperature. Discard flow-through and set the Spin Column CP4 back into the same collection tube.

   Note: Please load solution from step 7 to the Spin Column CP4 separately under above condition.

9. Add 500 μl Buffer PD to the Spin Column CP4 and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through and set the Spin

Column CP4 back into the same collection tube.

10.Add 600 μl Buffer PW **(Ensure that ethanol (96-100%) has been added)** to the Spin Column CP4 and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through and set the Spin Column CP4 back into the same collection tube.

Note: After the addition of Buffer PW, place the column at room temperature for 2-5 min would be helpful for the removal of contaminant.

11.Repeat the step 10.

12.Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to completely remove the residual buffer in the Spin Column CP4.

Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions (like enzyme digestion and PCR). We suggest open CP4 lid and stay at room temperature for a while to rid of residual ethanol.

13.To elute DNA, place the Spin Column CP4 into a clean microcentrifuge tube and add 100-300 μl Buffer TB to the center of the membrane and incubate 2 min at room temperature, centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Keep plasmid solution at the microcentrifuge tube.

Note: Transfer the plasmid solution back to the column and repeat step 12 would increase plasmid recovery efficiency. The pH value of elution buffer has significant influence on eluting; if distilled water is used to elute DNA, the pH value should be at 7.0-8.5. Low pH value (pH<7) would reduce the efficiency of eluting. If the volume of elution buffer is less than 100 μl, it may affect recovery efficiency. Extracted plasmid DNA should be stored at -20°C to avoid degradation

Analysis of yield and purity of plasmid DNA

The yield and purity of isolated plasmid DNA could be analyzed by agarose gel electrophoresis and UV spectrophotometry. Depending on the culturing time and the operation condition, there could be 1 or 2-3 bands on agarose gel. 1 value of OD_{260} corresponds to a 50 μg/ml dsDNA solution. $OD_{260/280}$ ratio value should be at 1.7-1.9, if distilled water was used to elute DNA, the value of $OD_{260/280}$ would be lower, but it does not mean that the purity of plasmid is lower since the pH value and ions would affect the light absorption value.